PC CODE: 006466 DP BARCODE: D252310 MRID 447343-06

DATA EVALUATION REPORT

Reviewer: Zig Vaituzis, Senior Scientist Date: 11/10/99
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (7511C)

Secondary Review by: Mike Mendelsohn Date: 11/10/99
Microbiologist
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (7511C)

STUDY TYPE: The study was conducted to compare the performance of male broiler chickens fed transgenic Bt Cry9C insecticidal, glufosinate resistant corn (CBH351) with a standard commercially available corn hybrid.

EPA GUIDELINES NUMBER: Not applicable. This is not a required study.

CITATION: Leeson, S. (1998) The effect of corn hybrid CBH351 on the growth of male broiler chickens. Department of Animal and poultry Science, Arkell Reserch Farms, University of Guelph, Guelph, Ontario, N1G 2W1 Canada. Lab project Id Number C-2-98. April 20,1998. MRID 447343-06

DP BARCODE: D252310 **CASE**: 290730

PETITION #: 9F05050 **CHEMICAL/BIOL#**: 006466 *Bacillus thuringiensis* subsp. tolworthi Cry9C protein

COMPANY/SPONSOR: (Submitter) AgrEvo USA Company, Little Falls Centre One, 2711 Centerville Rd.,Wilmington, De 199808.

TEST MATERIAL: *Bacillus thuringiensis* subsp. tolworthi Cry9C insecticidal, glufosinate resistant corn (event CBH351)

REVIEW CONCLUSION: This is not a required study and the results are inconclusive. Small differences were seen with the CBH351 test groups as compared to the the non-CBH351 test groups. Increased feed intake during the starter period, an increase in bird weight, and greater breast meat yield were observed. However, it is not possible to make an independent assessment of the significance of the data without an analysis of the bird feed for the presence of Cry9C protein.

RECOMMENDATIONS: None. This is not a required study and the results are inconclusive.

MATERIALS & METHODS: Test birds: Three hundred and sixty commercial strain Ross x Ross male broiler chickens obtained at (one) day of age were weighed and randomly distributed to 2 treatment groups, replicated 6 times, with 30 birds per replicate. The replicates were randomized over 12 floor pens each measuring 1.8 x 2.4 m. and cared for according to the guidelines of the

Canadian Council on Animal Care. The birds were reared on 1 of 2 diets (Bt- and Bt+ corn respectively, supplied by AgrEvo). The birds were fed starter diets to day 17 (57% corn as determined from raw data) , grower diets to day 31 (61% corn as determined from raw data) and finisher diets to day 42 (66% corn as determined from raw data).

Test conditions: the birds were maintained at a brooding temperature of 32° C for 5 days, with the temperature being gradually reduced to 22° C by day 33. The report states that constant lighting was used throughout the study, however, the supplied raw data show light periods diminishing from 23 hrs to 12 hrs at day 6, back to 18 hrs at day 14, then to 23 hrs at day 20 to end of study (day 42). Relative humidity was not reported.

Sampling: Feed intake and body weights were recorded at 17, 31 and 42 days. Mortality was recorded as it occurred.

Observations: The study was conducted from January 20, 1998 thru March 3, 1999. At day 42, eight randomly selected birds from each pen were sacrificed and the abdominal fat pad and the right and left breast meat were removed and weighed. No comments on the condition or appearance of the birds are given.

STATISTICAL METHODS: The data were considered by the T-test appropriate to 2 levels of treatment. Significance was accepted at $P < 0.05$.

REPORTED RESULTS: The 31 and 42 day (and 0-42 day) body weights of the birds fed Bt Cry9C, glufosinate resistant corn diets are significantly greater than the birds fed the control corn diet, and the body weight gain measured between days 17 and 31 is also significantly greater for the test birds. The actual difference in body weights was less than 3%. Feed intake for the Bt corn was significantly higher during the starter period (0-17 day). The reported mortality was 3.89% in the control group and 6.11% in the Bt corn group (not significantly different). Post mortem examination revealed a greater breast meat yield (<5%) for Bt fed birds. The feed intake/body weight ratios and percent mortality were unaffected by the source of corn in the diets. Carcass weight and abdominal fat pad and breast meat yield as a percent of carcass weight were also unaffected by the source of corn in the diet.

STUDY AUTHOR'S CONCLUSIONS: Bt Cry9C, glufosinate resistant corn (CBH351) diets are comparable in feeding value to the non-Bt corn hybrid. Actual body weight and breast meat yield was significantly different (<5% for all characteristics evaluated). Therefore in nutritive value CBH351 corn is at least equivalent to the commercial hybrid and did not adversely effect the feeding and growth of the male broiler population used in this study.

QUALITY ASSURANCE MEASURES: No Quality Assurance statement is included with the study report.

DISCUSSION: This was not a required study. In general the testing was conducted by acceptable procedures, and shows no significant mortality or acute effects from the consumption of Cry9C containing corn. However, analytical measurements were not performed as part of this study to

verify the homogeneity, stability or concentrations of the test substance in the test bird diet. This is a substantial deficiency, since small, but significant, differences were seen between the two test groups. Without the analysis of the bird diet it is not possible to make an independent assessment that the noted effects are due to CBH351 corn on the test birds.

The submitted data do show an effect on the feed intake during the starter period, a delayed effect on bird weight and a greater breast meat yield. A verification of the presence of Cry9C in the test diet is essential to determine which treatment group data are a result of Bt in corn.

It is necessary to have the diet analysis data to assess the significance of the reported results.

ADEQUACY OF STUDY: 1. Validation category: Not useful for avian diet effects assessment.
2. Rationale: The submitter does not claim GLP compliance, no Quality Assurance statement is included with the study report, and the report lacks analysis for the presence of Cry9C in the test diet.

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PC CODE: 006466 DP BARCODE: D240184 MRID 443843-01

EPA Reviewer: Zig Vaituzis, Ph.D. Date: 4/23/98
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division

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Secondary Reviewers:
Paul G. Forsyth, Ph.D. Signature: See last page
Robert H. Ross, M.S. Signature: See last page

Thru: Phil Hutton, Chief Date: 5/4/98
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (7511W)

DATA EVALUATION REPORT

STUDY TYPE: Non-Guideline Study

CITATION: MacIntosh, S.C. (1997) Preparation and characterization of catfish pellets. Plant Genetic Systems (America) Inc., 7200 Hickman Road, Ste. 202, Des Moines, IA. PGS Study No. 96QZM005, September 12, 1997. MRID 443843-01.

DP BARCODE: D240184
REG./FILE#: 070218-R
CASE: 061755
CHEMICAL/BIOL#: 006466 *Bacillus thuringiensis* subsp. *tolworthi* Cry9C protein

COMPANY/SPONSOR: Plant Genetic Systems, N.V., J. Plateaustraat 22, B-9000 Gent, Belgium.

TEST MATERIAL: Catfish pellets processed from corn kernels expressing the Cry9C gene incorporated from *Bacillus thuringiensis* subsp. *tolworthi*

REVIEW CONCLUSION: Based on results of a protein-specific ELISA analysis, no Cry9C protein was detectable in catfish pellets processed from corn kernels containing Cry9C protein.

RECOMMENDATIONS: None

ADEQUACY OF STUDY: Supplementary. This study was not intended to fulfill a guideline requirement.

MATERIALS & METHODS: The information in this study is not subject to GLP guidelines because the data are not necessary for product registration. A descriptive protocol (PGS 96QZM005) for the production of catfish pellets was included in the report. Catfish *Bt* pellets (Lot no. PEL-351-0196) were processed from transgenic corn kernels containing the CBH-351 event in a hemizygous form, i.e., containing the insecticidal Cry9C protein and the marker PAT (phosphinothricin acetyltransferase) which confers tolerance to phosphinothricin, the active ingredient in the herbicide, glufosinate-ammonium. Control material was catfish pellets (Lot no. PEL-351-0196C) processed from nontransgenic corn kernels. Reference material consisted of *Bt* whole corn kernels (Lot no. WCR-351-0196) and control whole corn kernels (Lot no. WCR-351-

0196C). Corn kernels processed to produce the test material were harvested from a plot containing a segregating population of corn (produced from a cross of hemizygous plants with wild type, therefore segregating 1:1 hemizygous to wild type). The nontransgenic plants were removed by treating the field with glufosinate-ammonium herbicide. The nontransgenic corn plants of the same line were harvested from another field.

Protein was extracted from test and control samples by milling in a blender and extracting according to SOP PGS-9H/0. Protein concentration was determined based on the Bradford protein quantification method (SOP PGS-9E/0). Cry9C content was analyzed by ELISA (Enzyme-Linked ImmunoSorbent Assay) according to SOP PGS-9D/2.

REPORTED RESULTS: The amounts of extractable protein from the *Bt* and control catfish pellets were 4.6 and 4.5 mg/g of pellet, respectively. *Bt* whole corn kernels contained 17.2 μg of Cry9C protein/g of kernel. No Cry9C protein could be detected in the *Bt* or control catfish pellets.

DISCUSSION: The study was not conducted in accordance with EPA GLP guidelines but was, according to the author, designed to meet the spirit of the guidelines. A draft protocol was present at the beginning of the study. Sample and reagent labeling, handling, and data entries did not meet guidelines but were either appropriate or corrected when possible.

Although the analysis part of the study was conducted at Plant Genetic Systems N.V. Belgium, no details on the place and time of growth of the corn plants or processing of the kernels into catfish pellets was given. Many procedures/protocols and raw data were not provided. This, however, is not expected to have any bearing on the results reported in the study.

REVIEWER'S COMMENTS:

A. **Test Procedures:** The procedures used are acceptable to the Agency.

B. **Statistical Analysis:** No analysis is needed since there was no detectable amount of toxin in the catfish food.

C. **Discussion/Risk Assessment**: The study is scientifically sound and no difference was observed between the processed and control replicates. Cry9C activity was shown to be destroyed following the extrusion procedure utilized in a typical fish food manufacturing process. Thus little or no Cry9C exposure to cultured fish is expected from Cry9C expressed in cord.

D. **Adequacy of the Study:**

1. Validation Category: Acceptable (non-guideline)

2. Rationale: This study adequately address potential toxicity concerns for cultured fish exposed to a diet containing Cry9C protein expressed in corn.

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DATA EVALUATION REPORT

CRY9C PROTEIN

STUDY TYPE: NON-GUIDELINE STUDY

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Crystal Station I
2800 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
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Oak Ridge, TN 37831

Task Order No. 21

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Disclaimer

This Data Evaluation Report may have been altered by the Biopesticides and Pollution Prevention Division subsequent to signing by Oak Ridge National Laboratory personnel.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464

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